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Letter to the Editors

Dose-independent kinetics with low level exposure to nicotine and cotinine

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In studying the effects of exposure to secondhand smoke (SHS), concentrations of cotinine (the proximate metabolite of nicotine), measured in plasma, urine or saliva, are widely used as indicators of nicotine exposure [1]. In interpreting the concentrations of cotinine in non-smokers relative to concentrations in smokers, the assumption is made that the pharmacokinetics of nicotine and cotinine are independent of the level of nicotine exposure.

Published pharmacokinetic data on nicotine and cotinine are based on administration of nicotine and cotinine to smokers and non-smokers in doses consistent with cigarette smoking, which are much higher than those relevant to SHS [2]. It is possible that the kinetics and metabolism of nicotine are different at low doses compared with higher doses. It is reported, for example, that the half-life of cotinine is longer after cessation of SHS exposure than after cessation of active smoking [3].

Nicotine is metabolized to cotinine primarily by the liver enzyme CYP2A6 [4]. The same enzyme also metabolizes cotinine to trans-3′-hydroxycotinine. The clearances of nicotine and cotinine and the activity of CYP2A6 are quite variable among individuals [5, 6]. Genetic variation explains some of the variability, with a number of *CYP2A6* gene variants known to be associated with reduced enzymatic activity resulting in slow metabolism of nicotine and cotinine [7]. The level of enzymatic activity could influence whether the nicotine clearance is dose-dependent in a particular person. For example, dose-dependence is more likely when enzyme activity low. For this reason, we also assessed *CYP2A6* genotype to examine its influence on dose-dependent pharmacokinetics.

We conducted a study of the disposition kinetics of nicotine and cotinine at doses that are consistent with intake of nicotine during SHS exposure. Sixteen healthy non-smokers (eight males, eight females) participated in a three dose randomized, crossover study. Subjects came to the clinical research centre at San Francisco General Hospital on three occasions during which they received intravenous infusions of deuterium labelled nicotine (nicotine-3′,3′-dideuteronicotine) and cotinine (cotinine-2,4,5,6-d_4), in doses of 0.05 μg kg^{-1}, 0.1 μg kg^{-1} and 0.2 μg kg^{-1}, infused over 60 min. Infusions were initiated at 07.00 h after an overnight fast, and blood samples were obtained over the subsequent 24 h. A blood sample was also obtained for genotyping for variants of the *CYP2A6* gene. Plasma nicotine concentrations were measured by GC-MS [8], modified for MS/MS determination using a triple quadrupole instrument to improve sensitivity. Cotinine was determined by LC-MS/MS [9]. Lower limits of quantitation were 0.1 ng ml^{-1} for both analytes. These subjects were genotyped for prevalent *CYP2A6* alleles of known impact on rates of metabolism, **2, *4H, *7- *10, *12, *17, *20, *23–26* and **35* [10–12]. Clearance, elimination half-life and steady-state volume of distribution were estimated using WinNonlin. The extent of conversion of nicotine to cotinine was estimated using the plasma concentrations of cotinine d2 (generated from nicotine d2), dose of nicotine d2 and the clearance of cotinine D4 using the following equation: $fc = (AUC_{COT-d2})/(dose_{nic-d2}) \times (CL_{COT-d4})$. Statistical comparison of pharmacokinetic analyses across different dose groups was conducted by two-way repeated measures analysis of variance, comparing doses of nicotine and genotype group (wild type *vs.* variant).

The 16 subjects averaged 34 years of age (range 20–56 years). Eight subjects were Caucasian, three Asian, three mixed race, one African American and one Hispanic. The disposition kinetics of nicotine and cotinine at the three doses are shown in Table 1. There were no significant differences by dose for any of the pharmacokinetic parameters. Eleven of the subjects had **1/*1 CYP2A6* genotype.

Table 1

Effect of nicotine dose on kinetics of nicotine and cotinine

Parameter	Dose Low	Middle	High
Nicotine			
All subjects			
$t_{1/2}$ (min)	129 (112, 146)	118 (102, 135)	123 (107, 140)
CL (ml min^{-1} kg^{-1})	14.2 (10.9, 17.5)	14.7 (11.4, 18.0)	15.7 (12.4, 19.0)
V_{ss} (l kg^{-1})	2.0 (1.7, 2.4)	2.0 (1.6, 2.4)	2.2 (1.9, 2.6)
By genotype (wild type, W *vs.* variant, V)			
$t_{1/2}$ (min)	W: 133 (110, 156)	W: 106 (84, 129)	W: 122 (100, 145)
	V: 124 (101, 150)	V: 131 (106, 155)	V: 125 (100, 149)
CL (ml min^{-1} kg^{-1})	W: 14.4 (9.9, 18.9)	W: 14.4 (9.9, 18.9)	W: 15.6 (11.1, 20.1)
	V: 14.0 (9.2, 18.8)	V: 15.1 (10.3, 19.9)	V: 15.8 (11.0, 20.6)
V_{ss} (l kg^{-1})	W: 2.1 (1.6, 2.6)	W: 1.8 (1.3, 2.3)	W: 2.2 (1.7, 2.7)
	V: 2.0 (1.4, 2.6)	V: 2.3 (1.7, 2.8)	V: 2.3 (1.7, 2.9)
Cotinine			
All subjects			
$t_{1/2}$ (min)	1006 (754, 1260)	998 (745, 1251)	1008 (754, 1261)
CL (ml min^{-1} kg^{-1})	0.54 (0.37, 0.72)	0.56 (0.35, 0.74)	0.57 (0.38, 0.77)
V_{ss} (l kg^{-1})	0.70 (0.56, 0.83)	0.70 (0.57, 0.84)	0.73 (0.60, 0.87)
f_c	0.70 (0.63, 0.77)	0.72 (0.65, 0.79)	0.73 (0.67, 0.80)
By genotype (wild type, W *vs.* variant, V)			
$t_{1/2}$ (min)	W: 916 (644, 1189)	W: 910 (637, 1182)	W: 910 (638, 1183)
	V: 1097 (813, 1382)	V: 1086 (802, 1370)	V: 1105 (821, 1389)
CL (ml min^{-1} kg^{-1})	W: 0.56 (0.37, 0.75)	W: 0.57 (0.38, 0.76)	W: 0.60 (0.41, 0.79)
	V: 0.52 (0.32, 0.72)	V: 0.55 (0.35, 0.74)	V: 0.57 (0.38, 0.77)
V_{ss} (l kg^{-1})	W: 0.66 (0.52, 0.81)	W: 0.66 (0.52, 0.80)	W: 0.69 (0.55, 0.83)
	V: 0.73 (0.58, 0.88)	V: 0.75 (0.60, 0.90)	V: 0.78 (0.63, 0.92)
f_c	W: **0.75 (0.68, 0.82)**	W: 0.74 (0.67, 0.82)	W: 0.76 (0.68, 0.83)
	V: **0.65 (0.57, 0.73)**†	V: 0.70 (0.62, 0.77)	V: 0.71 (0.63, 0.79)

Data are presented as mean (95% CI); $t_{1/2}$, elimination half-life; CL, systemic clearance, V_{ss}, steady-state volume of distribution, f_c, fractional conversion of nicotine to cotinine, W represents subjects with wild type and V with variant *CYP2A6* genes. †Significantly different ($P < 0.05$).

The remaining subjects had **1/*9* (two subjects), **1/*7*, **1/*12* and **1/*4H* (one subject each). Subjects with variant *CYP2A6* alleles had on average lower clearances of nicotine and cotinine and fractional conversion of nicotine to cotinine, but differences were not significant except for fractional conversion at the lowest dose of nicotine (Table 1). Among those with variant *CYP2A6* alleles, there was no evidence of dose-dependence differences in nicotine or cotinine pharmacokinetics.

Our study provides novel data on the pharmacokinetics of intravenous nicotine and cotinine dosed at levels that are consistent with exposure to SHS in non-smokers. We found no evidence of dose-related differences in nicotine and cotinine kinetics and found that the average pharmacokinetics were similar to those observed in smokers receiving higher doses of nicotine and cotinine [2, 6]. The presence of variant *CYP2A6* alleles was associated with slower metabolism of nicotine and cotinine as expected, but there was no evidence of dose-dependent kinetics in these subjects either.

In conclusion, we found that the disposition kinetics of nicotine and cotinine were similar at low levels of exposure compared with high levels of exposure, without evidence of dose-dependent kinetics. Cotinine concentrations measured in people with SHS exposure can be interpreted similarly to those of active smokers in estimating daily intake of nicotine.

Competing Interests

Dr Benowitz is a consultant to several pharmaceutical companies that market medications to aid smoking cessation and has served as a paid expert witness in litigation against tobacco companies. Dr Tyndale holds shares in Nicogen Research. No Nicogen funds were used in this work and others affiliated with Nicogen did not review the manuscript. Dr Tyndale has also been a paid consultant for Novartis and McNeil.

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